No 13

THE PHOTO-MICROGRAPHIC ROOM AND APPARATUS IN THE ANATOMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

By A. G. Hoen, M. D.

[From *The Johns Hopkins Hospital Bulletin*, Nos. 62-63, May-June, 1896.]

[From *The Johns Hopkins Hospital Bulletin*, Nos. 62-63, May-June, 1896.]

THE PHOTO-MICROGRAPHIC ROOM AND APPARATUS IN THE ANATOMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

By A. G. Hoen, M. D.

The scope of photo-micrography can conveniently be divided into photo-micrography (1) with high powers, (2) with medium powers, and (3) with low powers.

(1) Photo-micrography with high amplification (600 to 1200 diameters) is employed chiefly for the delineation of pathological material, such as micro-organisms, free and in sections of tissues, malarial and leukemic blood, etc., as well as for the demonstration of some of the minuter histological changes which occur in the cells, such as karyokinesis; the demonstration of nerve terminations in the tissues, as well as for the finer histological structures in general.

(2) Photo-micrography with medium amplification (150 to 400 diameters) is applied to tissue work, where it is desirable to differentiate structural elements in pathological as well as in normal histological material, micro-urinary deposits, and the study of vital movements (amœboid) of certain cells by serial exposures. Under this heading it is perhaps well to enter our criticism in reference to the illustrations as found in the various text-books on normal histology. Beautifully as the majority of them are executed, we cannot help but point out their deficiency in properly conveying to the student's mind the correct appearance of the microscopic image or field. It has become a fixed fact in the minds of instructors that teaching histology from drawings and diagrams is by no means altogether satisfactory. Most of the diagrams are selected from exceptionally fine or lucky specimens, and many of them are composite pictures, taken from different slides, the salient points of one being blended with those of others, thus making perfect pictures which have a tendency to puzzle

the student, because he cannot verify them from his sections. The diagrams are intended to elucidate the subject, and if they are accompanied with photographs they aid markedly in giving the student the proper impression of the subject at hand, nothing more or less; whilst on the other hand a drawing, no matter how carefully and conscientiously conducted, has constantly mixed with it a certain element, individualism, personal equation, if one chooses, which cannot be eliminated or ruled out. Furthermore the draughtsman never thinks of portraying the imperfections, bubbles of air, dust, cotton fibers, etc., which are likely to be present, even in the best preparations. The very perfection of his drawing is misleading and incorrect.

With a view to eliminating these difficulties, we have made in connection with the histological course, a series of well executed photo-micrographs, taken from the best specimens as prepared and stained by the students themselves. From these photographs lantern slides have been made for projection on a screen. For lower powers than 150 diameters, the specimen itself is utilized for projection by a system of lenses to be described further on.

(3) Photo-micrographs with low powers (from 1 to 100 diameters) applied for the purposes of studying various tissues with reference to their anatomical relations and for injected specimens of whole organs. Also extremely useful in embryological research, in which it has been found of great service as a time-saver to give correct outlines for reconstructions. The above uses of photo-micrography have been kept in mind in purchasing and elaborating the apparatus in the Anatomical Laboratory. Primarily the apparatus was intended for scientific investigation, but in the course of time it was found extremely useful for teaching as well. We shall describe our apparatus under the following heading:

The Rooms.—In constructing the rooms we were guided by the idea that the operator should work within the camera, so we made the main room perfectly dark. This communicates with a room for illuminating purposes on one hand and with a second dark room for developing on the other hand. Of these three rooms, the larger one contains the tables carry-

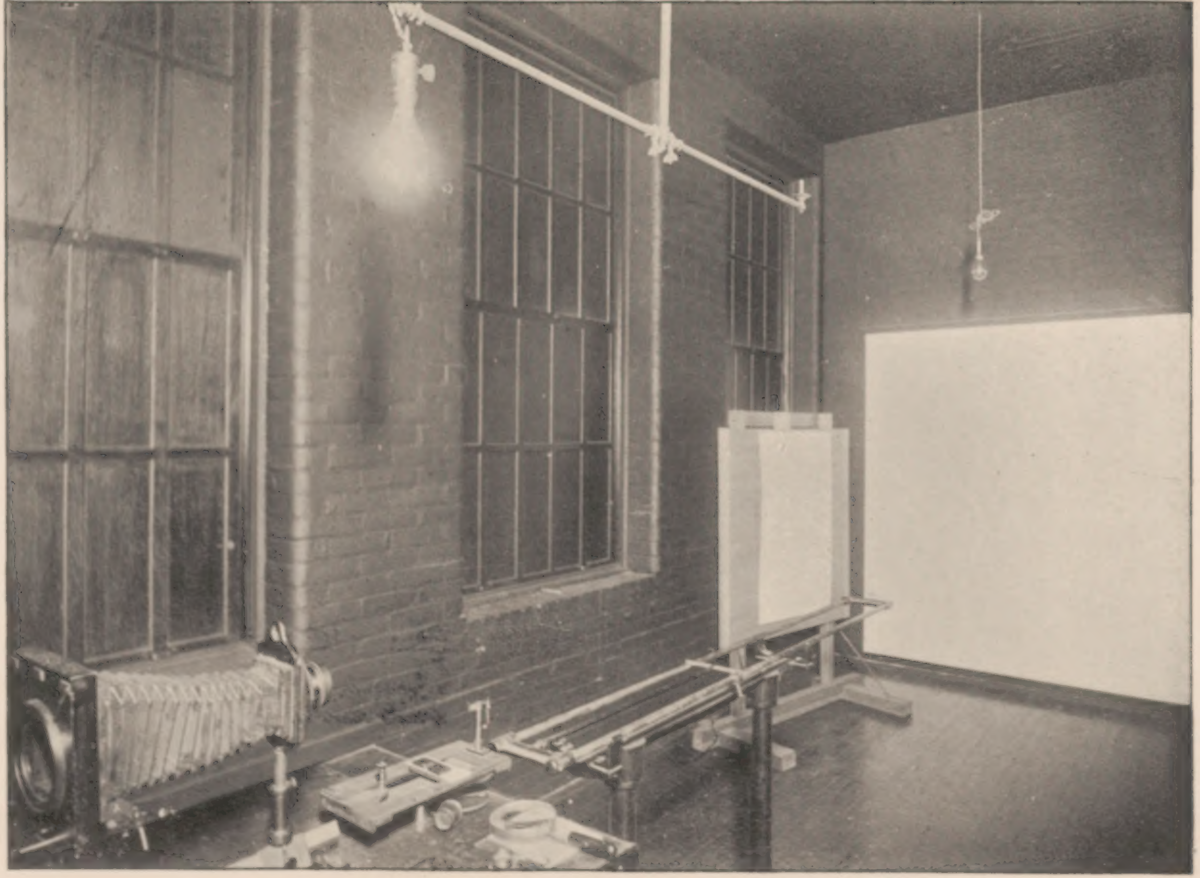

FIG. 3. NORTH-WEST CORNER OF THE PROJECTING ROOM.

The permanent screen is attached to the wall for ordinary projecting work with either high or low powers. The movable screen is employed when a definite number of diameters are desired, as in making charts, bromides, or simple outline drawings upon wax plates. On the left of the figure is the magic lantern adjustment attached to the Zeiss stand.

Fig. 2. South-west Corner of the Projecting Room.

Flash-light photograph. The light is in the next room as indicated in the ground floor plan.

ing the condensers, microscope, camera and projecting apparatus, whilst the smallest, well lighted one contains the electric arc light and tables for ordinary microscopic observation. The second dark room contains the necessary chemicals for developing plates and conducting such other manipulations as appertain to photography. It is supplied with hot and cold water and is lighted by incandescent lamps, one of which is hooded with black velvet over a frame of asbestos and the lower end is covered by three thicknesses of deep ruby glass. The accompanying figure shows the floor plan of these rooms with some of the apparatus in place. The walls and ceiling of the large room are painted a dull or flat black from which no reflection can take place. The windows are protected against the admission of light by venetian blinds on the outside, an adjustable black cloth one on the inside, and over these, sliding paneled wooden blinds. The inside blinds are also painted black, and are provided with screw clamps for taking up warps in the wood and to ensure perfect contact of their surfaces.

The table bearing the condensers, light filter and microscope is so placed that it directly faces the aperture in the partition, through which the light from the electric arc lamp is projected by the paraboloid reflector to be mentioned further on. The table bearing the camera is so placed that it forms a continuation of the first; both tables are brought into the same plane by means of set-screws.

The tables and camera were made by Zeiss, but we found it necessary to add these set-screws to adjust them to a given plane easily. On the north wall, directly opposite the opening in the partition, a plaster of Paris screen has been made, which is perfectly smooth to ensure a good reflecting surface. This screen is employed to receive the projected image, with low powers, for demonstrating histological specimens themselves to the classes. In addition to this it was found necessary to have a smaller movable screen constructed, which is also adjustable vertically, its use being to adjust the apparatus easily to any given number of diameters in making diagrams and reconstructions.

Illumination.—Sunlight is perhaps the one source of illumination that every photo-micrographer has tried some time in the course of his work, and has been glad to abandon for any new illuminant which would give him practically the same actinic power. There are, however, so many drawbacks to it that it is necessary to observe the time of the year, the day, the hour and the condition of the atmosphere as factors which determine the power of his light, not to say anything of the many disappointing days when it does not shine at all. In our laboratory sunlight has been entirely superseded by the electric arc light, with which we are capable of accomplishing everything desired. The lamp used is the ingenious invention of Coerper, of Ehrenfeld, Germany, and manufactured in America by the Helios Electric Light Co. of Pennsylvania.* The lamp we employ is calculated to be of 4000 candle power, and is run by an alternating current of 30 amperes, and is in no respect dangerous to life. The construction is extremely simple. A glance at Fig. 4 shows the carbon holders balanced by means of a chain over a pulley in the lamp mechanism. As a result of this arrangement with the alternating current, the combustion of the carbons is compensated for by the descent of the upper and the ascent of the lower carbon in exactly the same ratio, and the arc is thus fixed practically at one point, and remains there from the time the current is turned on until the carbons are consumed. We found it necessary to substitute for the upper porcelain reflector one made in the form of a paraboloid, which completely encloses both carbon points. Our reflector is made of one piece of copper, lined on the inside with a thick coating of white lime, and our experience with it convinces us that it has very markedly increased the power of the light. In order to easily adjust the lamp in the optical axis of our photo-micrographic apparatus, we adopted the plan of mounting it on a mechanical stage, made much after the pattern of that employed on the better class of microscopes. This frame is attached to the wall in an upright position, thus giving both vertical and lateral movements, and we find that it works admirably, and is capable of adjusting the lamp to the frac-

* McKay-Howard Electric Construction Co., Baltimore, Md.

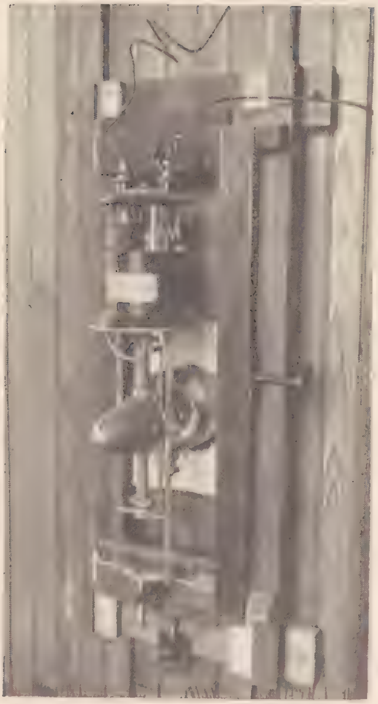

Fig. 4. Helios Electric Lamp Attached to the North Wall of the Observation Room.

The position of the lamp is indicated by the * on the ground floor plan.

tion of an inch. These alterations and modifications were made by Messrs. Murrill & Keizer of Baltimore, to whom we are very much indebted for advice and suggestions in reference to the same. In attaching the parabolic reflector to the lamp, the carbon points were made to pass through two openings in it in order to keep them in place. Experience showed us, however, that there was considerable vibration of the two points in spite of this, which we overcame by attaching a guard to the side of the lamp, to steady the upright rod of the lower carbon point. This guard is adjustable with a screw, as the figure shows. With these adjustments we succeeded in attaining a source of light which is not continually moving up and down, as in the case of the lamp furnished by Zeiss. The heat generated by this lamp is considerable, and it was found necessary to protect the mechanical stage and the surrounding woodwork by asbestos sheeting.

Apparatus and Accessories.—The apparatus consists of a Zeiss stand I^{a} for photo-micrography with 2, 2.5, 4, 8, 16, 35, and 70 mm. lenses and Nos. 2 and 4 projection oculars. In addition to these lenses, there are also the following photographic lenses which can be used for photographing with low powers as well as for ordinary lantern projection:

Anastigmat	1: 6.3,	focus	43	millimeters.
	1: 7.2,	"	96	"
	1: 7.2,	"	148	"
	1: 12.5,	"	260	"
	1: 18,	"	632	"

With these lenses it is possible to photograph objects from one diameter upwards, and this is very necessary, for it is often desired to obtain a specific enlargement of a specimen, as in reconstruction work.

In addition to these lenses there is a complete projection table with its appendages, as well as the large camera. For delicate work there is a spectral illuminating apparatus after Hartnack.

The anastigmat lenses are all interchangeable, and the large ones are fitted with a prism to photograph at right angles to the object, as is often necessary in photographing objects under fluids. The sub-stage of the condenser of the microscope is that constructed after the formula of Prof. Abbé, is achro-

matic and supplied with two iris diaphragms, by which the access and egress of light may be regulated to the requirements of the amplification to be used, or in other words, they permit the use of the entire aperture of the condenser or only fractional portions thereof, as the case may be. It is fitted with two adjusting screws, by which means the condenser may be accurately centered for the objective in use, and an even and uniform illumination of the field secured.

As two tables or stands are supplied with this apparatus, one bearing the condensers, light filter and sole plate for the microscope, and the other the camera, we found that it was impossible to bring both tables to an exact level with each other or to retain them there, in consequence of which it was also impossible to establish a perfect optical line between the former and the latter. We found an adequate remedy for this by having set-screws put into each one of the iron pedestals of the tables; these screws rest in small metallic discs, which are simply laid on the floor in their proper position. This improvement of the Zeiss table has been found very serviceable in leveling the tables from time to time.

Methods of Illumination for High and Medium Powers.—1st Method: In order to illustrate this it is necessary to refer to Fig. 1. The star represents the arc light, the rays of which are received upon the plano-convex lens *x*, which is so placed that the arc is in its principal focus, thus rendering all rays passing through it, parallel. In their further course through the bi-convex lens *xx* the rays are brought to a focus, which is made to coincide, by adjusting this lens on the sliding bar, with the principal focus of the lower lens of the Abbé condenser in the microscope (*M*). The lower lens of the Abbé condenser renders the rays parallel, while its second lens converges them and brings them to a focus in the plane of the object, giving there a small but very bright image of the source of illumination.

2d Method: This consists in the use of a plano-convex lens of much shorter focus than the one supplied with the outfit. It was made for us by Messrs. Bausch & Lamb of Rochester, N. Y., and is mounted in a metal frame which is fastened to the wall, and by means of set-screws permits of perpen-

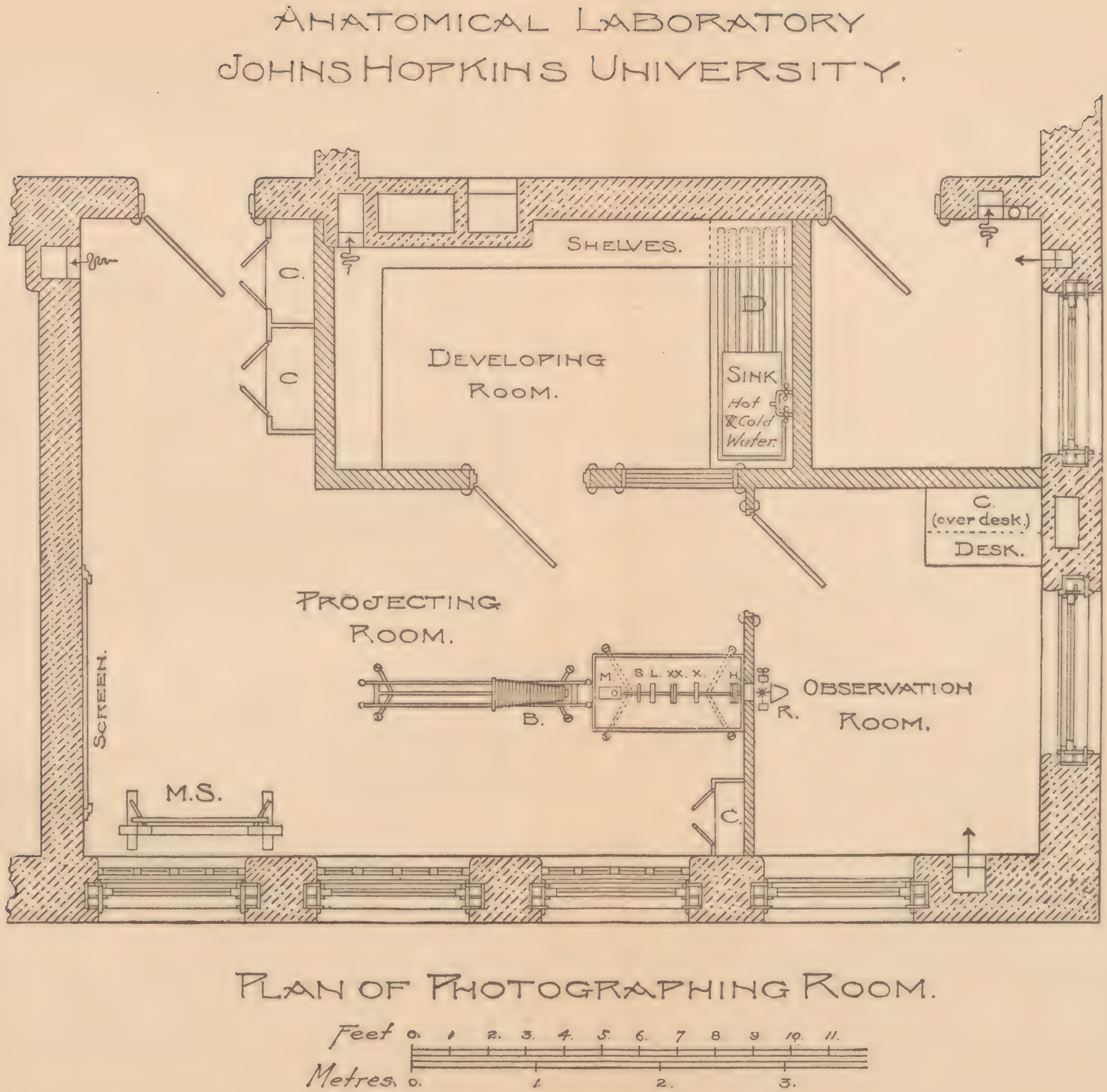

FIG. 1.

C, Cases. *D*, Drip-board. *MS*, Movable screen. *B*, Bellows. *M*, Microscope. *S*, Specimen holder. *L*, Light filter. *xx*, Double convex lens. *x*, Plano-convex lens. *H*, Heat filter. *, Electric light. *R*, Reflector.

dicular as well as lateral movements. The collar is also movable in the optical axis, so that the distance between the collecting lens and the light can be regulated with ease. The lens is four inches in diameter and its principal focal distance is five inches. Its adjustment for accurately focusing the lamp is accomplished by means of the set-screw, which is moved back and forth until a bright beam of light, not greater in diameter than the lens itself, is projected on the screen. Fig. 4 of the lamp shows the lens as having been pushed through the opening in the partition by means of the set-screw and in position for focusing the lamp.

In order to accurately center the Abbé condenser for the objective system we have adopted the following plan: The condenser is racked down far enough to enable us to unscrew its top cap which carries the small, nearly hemispherical and the upper achromatic plano-convex lenses of the system. This discloses the iris diaphragm situated immediately below it, the aperture of which is now reduced to its smallest diameter by the regulating lever of this part of the condenser. The resulting small opening is now carefully brought into the focus of a weak objective (16 mm.) and a low eyepiece (No. 4), and by means of the centering screws attached to the condenser, lateral and vertical movements are imparted to the latter until the opening in the diaphragm occupies a central position in the visual field of the microscope. We may state here that all of our objectives are carefully centered to the optical axis by Zeiss' "sliding objective changer."

To center the source of illumination to the optical axis the following was found to be the speediest and most certain method in our hands: The lenses of the guide-bar as well as those of the microscope, including the Abbé condenser and eyepiece, are removed; a pin-hole diaphragm is now placed in an eyepiece (freed of its lenses), which is slipped into the tube of the microscope. By looking through the pin-hole opening in the blank eyepiece, the carbon points of the electric arc light (without current) can now be brought into view by means of the adjusting screws on the mechanical stage bearing the lamp, to occupy a position corresponding to the opening in the diaphragm in the eyepiece. Once so adjusted the lamp has a tendency to remain in this position, requiring perhaps one adjustment in the course of six hours use.

The lenses for rendering the light parallel and condensing it are now placed upon the guide-bar and so adjusted that a sharp and bright image of the burning carbons is projected exactly in the center of the lower iris diaphragm of the Abbe condenser, which is closed to its utmost for this purpose.

For very high powers (immersion systems) the whole aperture of the Abbé condenser is utilized by opening the iris diaphragms to their fullest extent; for medium powers about one half of this aperture is sufficient. We found in practice that the illumination of the object which gave the most clear and sharp picture visually also answered the best purposes for photography. To secure the best illumination, the condensing lens *xx* is moved back and forth upon the guide-bar until the projected image of the microscopic field upon the ground glass of the camera is brightest and most evenly illuminated, thus fixing the position for the lens *xx*, for the focus of this lens and of the lower lens of the Abbe condenser are now coincident.

Light Filter.—We have confined ourselves exclusively to the bichromate of potash and sulphate of copper solution as recommended by Neuhaus in his admirable work on Photomicrography (p. 64, 1890). It is made as follows:

Sulphate of copper, 175 grams.
Bichromate of potash, 17 grams.
Sulphuric acid, 2 cc.
Water, from 500 to 1000 cc.

The more concentrated solution is applicable to specimens stained very lightly with the various blues (haematoxylon, anilines, etc.) or for the reds, particularly safranin preparations. Eosin is a decidedly disturbing element in photomicrography, and unless it is very cautiously and lightly used, as a counter-stain to other dyes, produces an indefinable haze or indistinctness upon the plate which mars an otherwise good negative very much. As practically no heat rays permeate this solution, it answers the purpose of a heat filter thoroughly. The position of the light filter is of little moment, provided that the rays of light which pass through it are the only ones which reach the Abbé condenser.

Focusing.—With a camera length of 55 to 60 cm. we have rarely found it necessary to have recourse to any special focusing appliance; the fine adjustment is within reach of the hand, while watching the appearance of the image on the ground glass. The focusing glass we employ is an ordinary hand lens, with which most of our work requiring from 150 to 400 diameters is accomplished.

The plate for intercepting the image is made of finely ground glass, such as Zeiss supplies with his outfit. For bacterial work a plane glass screen is used in connection with Zeiss' focusing lens, which is adjustable to the eye of the operator.

When the long camera is employed we use the focusing rod and Hooke's key as furnished with the Zeiss outfit.

Plates.—After using the plates of a number of manufacturers, we have selected those made by the "Cramer Dry Plate Company" of St. Louis, Mo., as those which give us uniformly the best results.

They are orthochromatic in the widest sense, are very uniform, and the instantaneous or extra rapid plates made by this company are sensitive to an exquisite degree.* For bacterial work we use the latter (1000 to 1200 diameters), preferring the "medium" plates for histological purposes (150 to 400 diameters).

The development of these plates is accomplished as advised by, and after the formula of the manufacturers (in their circular), by a combination of hydroquinone and eikonogen. Care is necessary in manipulating these plates in the dark room with reference to the light used, which must be of a deep ruby red and feeble, otherwise they will fog. It is safer to develop the plates away from the light, using the latter only occasionally to watch the progress of the development.

Determination of Amplification and Means of Measurement.—With the Zeiss system of objectives and projection oculars,

---

* From two to five seconds exposure is sufficient to impress these plates, even under such great dispersion of light as takes place, for instance, with the 2 mm. immersion system of 1.40 N. A. The advantages of this short exposure are obvious.

we have adopted the plan as advocated by him in his "Special Catalogue," (p. 36), and also noted in Neuhaus' work before referred to (p. 72).

As Zeiss has adopted a nomenclature for his objectives and oculars by designating them by their focal distances, it is an easy matter to arrive at the magnifying power of any one combination of objective and projection ocular by having recourse to the following formula:

$\frac{L}{O} \times P = x$ — in that L represents the camera length, O the focal distance of the objective, and P that of the projection ocular.

If therefore an objective of 2 mm. focal distance and the projection ocular No. 4 be used in combination with a camera length of 550 mm., we shall have:

$$\frac{550}{2 \text{ mm.}} \times 4 = \frac{1100}{1} \text{ linear.}$$

In this calculation the reckoning must be made from the shoulder of the ocular to the ground glass or screen. In those instances where it is necessary to be extremely accurate in the measurements, the stage micrometer ruled in 10ths and 100ths of a millimeter is made use of.

Yet for accurate work it is always necessary to control by projecting a millimeter scale. These we have had ruled in square millimeters, in square tenths and hundredths. For careful reconstruction work this is very necessary, as a projected rule shows easily any irregularity in the amplification at the periphery of the field.